

1/12

Ca	23					S	AFNTQAAP	31
Cs	1					G		1
Bp	1	RTITNNEMGN	HSGYDYELWK	DYGNT-SMTL	NNGGAFSAGW	N--NIGNA		45
Ca	32	KTITSNEIGV	NGGYDYELWK	DYGNT-SMTL	KNGGAFSCQW	S--NIGNA		76
Cs	2	RIIYDNETGT	HGGYDYELWK	DYGNT-IMEL	NDGGTFSCQW	S--NIGNA		46
Rf	1	SAADQQTRGN	VGGYDYEMWN	QNGQGQASMN	PGAGSFTCSW	S--NIENF		46
Tr2	1	QTIQPGTGY	NNGYFYSYWN	DGHGGVITYN	GPGGQFSVNW	S--NSGNF		45
Tv	1	QTIQPGTGF	NNGYFYSYWN	DGHGGVITYN	GPGGQFSVNW	S--NSGNF		45
Th	1	QTIGPGTGY	SNGYYSYWN	DGHAGVITYN	GGGGSFTVNW	S--NSGNF		45
Sc	1	SGTPSSTGT	DGGYYSWWT	DGAGDATYQN	NGGGSYTLTW	SG--NNGNL		46
An	1		S	AGINYVQNYN	GNLGDFTY-D	ESAGTFSMYW	EDGVSSDF	38
AT	1		S	AGINYVQNYN	QNLGDFTY-D	ESAGTFSMYW	EDGVSSDF	38
Tr1	1			ASINYDQNYQ	TGG-QVSY-	PSNTGFSVNW	N--TQDDF	34
Ss	1	ATTIT-NETGY	D-GMYYSFWT	DGGGSVSMTL	NGGGSYSTRW	T--NCGNF		45
SlB	1	DTVVTTNQEGT	NNGYYSFWT	DSQGTVSMNM	GSGGQYSTSW	R--NTGNF		47
SlC	1	ATTITTNQTGT	D-GMYYSFWT	DGGGSVSMTL	NGGGSYSTQW	T--NCGNF		46
Tf	1	AVTSNETGY	HDGYFYSFWT	DAPGTVSMEL	GPGGNYSTSW	R--NTGNF		45
Bc	1			ASTDYWQNWT	DGGGIVNAVN	GSGGNYSVNW	S--NTGNF	36
Bs	1			ASTDYWQNWT	DGGGIVNAVN	GSGGNYSVNW	S--NTGNF	36
Bp	46	LFRK-GKKFD	ST-RTHHQLG	NISINYNASF	N-PSGNSYLC	VYGTQSP		90
Ca	77	LFRK-GKKFN	DT-QTYKQLG	NISVNYNCNY	Q-PYGNSYLC	VYGTSSP		121
Cs	47	LFRK-GRKFN	SD-KTYQELG	DIVVEYGCDY	N-PNGNSYLC	VYGTTRNF		91
Rf	47	LARM-GKNYD	SQKKNYKAFG	NIVLTYDVEY	T-PRGNSYMC	VYGTTRNP		92
Tr2	46	VGGK-GWQPG	TKNKV-----	---INFS-GS	YNPNGNSYLS	VYGWSRNP		83
Tv	46	VGGK-GWQPG	TKNKV-----	---INFS-GS	YNPNGNSYLS	VYGWSRNP		83
Th	46	VGGK-GWQPG	TKNKV-----	---INFS-GS	YNPNGNSYLS	IYGWSRNP		83
Sc	47	VGGK-GWNP	AASRS-----	---ISYS-GT	YQPNGNSYLS	VYGTTRSS		84
An	39	VVGL-GWTTG	SSNA-----	---ITYSAEY	SASGSSSYLA	VYGVNYP		76
At	39	VVGLGGWTTG	SSNA-----	---ITYSAEY	SASGSASYLA	VYGVNYP		77
Tr1	35	VVGW-GWTTG	SSAP-----	---INFGGSF	SVNSGTGLLS	VYGWSTNP		72
Ss	46	VAGK-GWANG	GR-RT-----	---VRYT-GW	FNPSGNGYGC	LYGWTSNP		82
SlB	48	VAGK-GWANG	GR-RT-----	---VQYS-GS	FNPSGNAYLA	LYGWTSNP		84
SlC	47	VAGK-GWSTG	DGN-----	---VRYN-GY	FNPVGNGYGC	LYGWTSNP		82
Tf	46	VAGK-GWATG	GR-RT-----	---VTYS-AS	FNPSGNAYLT	LYGTTRNP		82
Bc	37	VVGK-GWTTG	SPFRT-----	---INYNAGV	WAPNGNGYLT	LYGTTRSP		75
Bs	37	VVGK-GWTTG	SPFRT-----	---INYNAGV	WAPNGNGYLT	LYGTTRSP		75

FIGURE 1

2/12

Bp 91 LAEYYIVDSW GTYR-PT--G AYKGSFYADG GTYDIYETTR VNQPSIIG 135
 Ca 122 LVEYYVIDSW GSWRPP--GG TSKGTITVDG GIYDIYETTR INQPSIQG 167
 Cs 92 LVEYYIVESW GSWRPP--GA TPKGTITQWMAGTYEYIYETTR VNQPSIDG 138
 Rf 93 LMEYYIVEGW GDWRPPGNDG EVKGTVSANG NTYDIRKTMR VNQPSLDG 140
 Tr2 84 LIEYYIVENF GTYN-PSTGA TKLGEVTSBG SVYDIYRTQR VNQPSIIG 130
 Tv 84 LIEYYIVENF GTYN-PSTGA TKLGEVTSBG SVYDIYRTQR VNQPSIIG 130
 Th 84 LIEYYIVENF GTYN-PSTGA TKLGEVTSBG SVYDIYRTQR VNQPSIIG 130
 Sc 85 LIEYYIVESY GSYD-PSSAA SHKGSVTCNG ATYDILSTWR YNAPSIDG 131
 An 77 GAEYYIVEDY GDYN-PCSSA TSLGTVYSDG STYQVCTDTR INEPSITG 123
 At 78 QAEYYIVEDY GDYN-PCSSA TSLGTVYSDG STYQVCTDTR INEPSITG 124
 Tr1 73 LVEYYIMEDN HNY--PAQ-G TVKGTVTSDG ATYTIWENTR VNEPSIQG 117
 Ss 83 LVEYYIVDNW GSYR-PT--G ETRGTVHSDG GTYDIYKTTR YNAPSVEA 127
 SlB 85 LVEYYIVDNW GTYR-PT--G EYKGTVTSDG GTYDIYKTTR VNKPSVEG 129
 SlC 83 LVEYYIVDNW GSYR-PT--G TYKGTVSSDG GTYDIYQTTR YNAPSVEG 127
 Tf 83 LVEYYIVESW GTYR-PT--G TYMGTVTTDG GTYDIYKTTR YNAPSIEG 127
 Bc 76 LIEYYVDSW GTYR-PT--G TYKGTVKSDG GTYDIYTTTR YNAPSIDG 120
 Bs 76 LIEYYVDSW GTYR-PT--G TYKGTVKSDG GTYDIYTTTR YNAPSIDG 120

Bp 136 -IATFKQYWS VROTKRTS-- -----GTVS VSAHFRKWES LGMPM-GK 174
 Ca 168 -NTTFKQYWS VRRTKRTS-- -----GTIS VSKHFAAWES KGMPM-GK 206
 Cs 139 -TATFQYWS VRTSKRTS-- -----GTIS VTEHFPOWER MGMRM-GK 177
 Rf 141 -TATFQYWS VROTSQSANN QTNMKGITD VSKHFDASAS AGLDMSGT 187
 Tr2 131 -TATFQYWS VRRNHR-S-S -----GSVN TANHFNAWAQ QGLTL-GT 168
 Tv 131 -TATFQYWS VRRTHR-S-S -----GSVN TANHFNAWAQ QGLTL-GT 168
 Th 131 -TATFQYWS VRRNHR-S-S -----GSVN TANHFNAWAS HGLTL-GT 168
 Sc 132 -TQTFEQFWS VRNPKKAPGG SIS---GTVD VQCHFDWKG LGMNLGSE 175
 An 124 -TSTFTQYFS VRESTRTS-- -----GTVT VANHFNFWAQ HGFNG-SD 162
 At 125 -TSTFTQYFS VRESTRTS-- -----GTVT VANHFNFWAH HGFHN-SD 163
 Tr1 118 -TATFNQYIS VRNSPR-T-S -----GTVT VQNHFN-WAS LGLHLGQM 155
 Ss 128 -PAAFDQYWS VRQSKVT--S -----GTIT TGNHFDWAR AGMNMGNF 168
 SlB 130 TR-TFDQYWS VRQSKR-TG- -----GTIT TGNHFDWAR AGMPLGNF 168
 SlC 128 TK-TFQYWS VRQSKVTSGS -----GTIT TGNHFDWAR AGMNMGQF 168
 Tf 128 TR-TFDQYWS VRQSKRTS-- -----GTIT AGNHFDWAR HGMHLGTH 166
 Bc 121 DRTTFTQYWS VRQSKRPTGS N-----ATIT FTNHVNAWKS HGMNLGSN 163
 Bs 121 DRTTFTQYWS VRQSKRPTGS N-----ATIT FSNHVNAWKS HGMNLGSN 163

FIGURE 1 (CONT'D)

3/12

Bp	174	MYETAFTVEG	YQSSGSANVM	TNQLFIGN	201
Ca	207	MHETAFNIEG	YQSSGKADVN	SMSINIGK	233
Cs	178	MYEVALTVEG	YQSSGYANVY	KNEIRIGANP....	
Rf	188	LYEVSLNIEG	YRSNGSANVK	SVSV	211
Tr2	169	MDYQIVAVEG	YFSSGSASI-	TVS	190
Tv	169	MDYQIVAVEG	YFSSGSASI-	TVS	190
Th	169	MDYQIVAVEG	YFSSGSASI-	TVS	190
Sc	176	HNYQIVATEG	YQSSGTATI-	TVT	197
An	163	FNYQVMAVEA	WSGAGSASV-	TISS	184
At	164	FNYQVMAVEA	WSGAGSAAV-	TISS	185
Tr1	156	MNYQVMAVEG	WGGSGSASQ-	SVSN	178
Ss	167	RYYMINATEG	YQSSGSSTI-	TVSG	189
SlB	169	SYMINATEG	YQSSGTSSI-	NVGG.....	
SlC	169	RYYMINATEG	YQSSGSSNI-	TVSG	191
Tf	167	D-YMIMATEG	YQSSGSSNVT	LGTS.....	
Bc	164	WAYQVMATEG	YQSSGSSNV-	TVW	185
Bs	164	WAYQVMATEG	YQSSGSSNV-	TVW	185

Bp	<i>Bacillus pumilus</i>
Ca	<i>Clostridium acetobutylicum</i> P262 XynB
Cs	<i>Clostridium stercorarium</i> xynA
Rf	<i>Ruminococcus flavefaciens</i>
Tr2	<i>Trichoderma reesei</i> XYN II
Tv	<i>Trichoderma viride</i>
Th	<i>Trichoderma harzianum</i>
Sc	<i>Schizophyllum commune</i> Xylanase A
An	<i>Aspergillus niger</i> , var. <i>awamori</i>
At	<i>Aspergillus tubigensis</i>
Tr1	<i>Trichoderma reesei</i> XYN I
Ss	<i>Streptomyces</i> sp. 36a
SlB	<i>Streptomyces lividans</i> Xln B
SlC	<i>Streptomyces lividans</i> Xln C
Tf	<i>Thermomonospora fusca</i> TfxA
Bc	<i>Bacillus circulans</i>
Bs	<i>Bacillus subtilis</i>

FIGURE 1 (CONT'D)

4/12

5'-CT AGC TAA GGA GG CTG CAG ATG
G ATT CCT CC GAC GTC TAC
NheI | PstI

TrX-1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Q T I Q P G T G Y N N G Y F Y S
CAA ACA ATA CAA CCA GGA ACC GGT TAC AAC AAC GGT TAC TTT TAC AGC
GTT TGT TAT GTT GGT CCT TGG CCA ATG TTG TTG CCA ATG AAA ATG TCG
TrX-8 AgeI

XyTv-2
17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
Y W N D G H G G V T Y T N G P G
TAT TGG AAC GAT GGC CAT GGT GGT GTT ACC TAT ACA AAC GGG CCC GGA
ATA ACC TTG CTA CCG GTA CCA CCA CAA TGG ATA TGT TTG CCC GGG CCT
NcoI XyTv-7 ApaI

33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
G Q F S V N W S N S G N F V G G
GGC CAA TTT AGC GTC AAT TGG TCT AAC TCC GGA AAC TTC GTA GGT GGA
CCG GTT AAA TCG CAG TTA ACC AGA TTG AGG CCT TTG AAG CAT CCA CCT
MunI BspEI

TrX-3
49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64
K G W Q P G T K N K V I N F S G
AAA GGT TGG CAA CCC GGG ACC AAA AAT AAG GTG ATC AAC TTC TCT GGA
TTT CCA ACC GTT GGG CCC TGG TTT TTA TTC CAC TAG TTG AAG AGA CCT
XmaI TrX-6

65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80
S Y N P N G N S Y L S V Y G W S
TCT TAT AAT CCG AAT GGG AAT TCA TAC TTA AGC GTC TAT GGC TGG TCT
AGA ATA TTA GGC TTA CCC TTA AGT ATG AAT TCG CAG ATA CCG ACC AGA
EcoRI AflII

XyTv-4
81 82 83 84 85 86 87 88 89 90 91 92 93 94 95
R N P L I E Y Y I V E N F G T
AGA AAC CCA CTG ATT GAA TAT TAC ATT GTC GAA AAT TTC GGT AC
TCT TTG GGT GAC TAA CTT ATA ATG TAA CAG CTT TTA AAG C
XbaI KpnI

FIG. 2

5/12

[illegible]

FIG. 2 (CONT'D)

6/12

Thermostability of mutant TrX

Incubation at 62.5°C

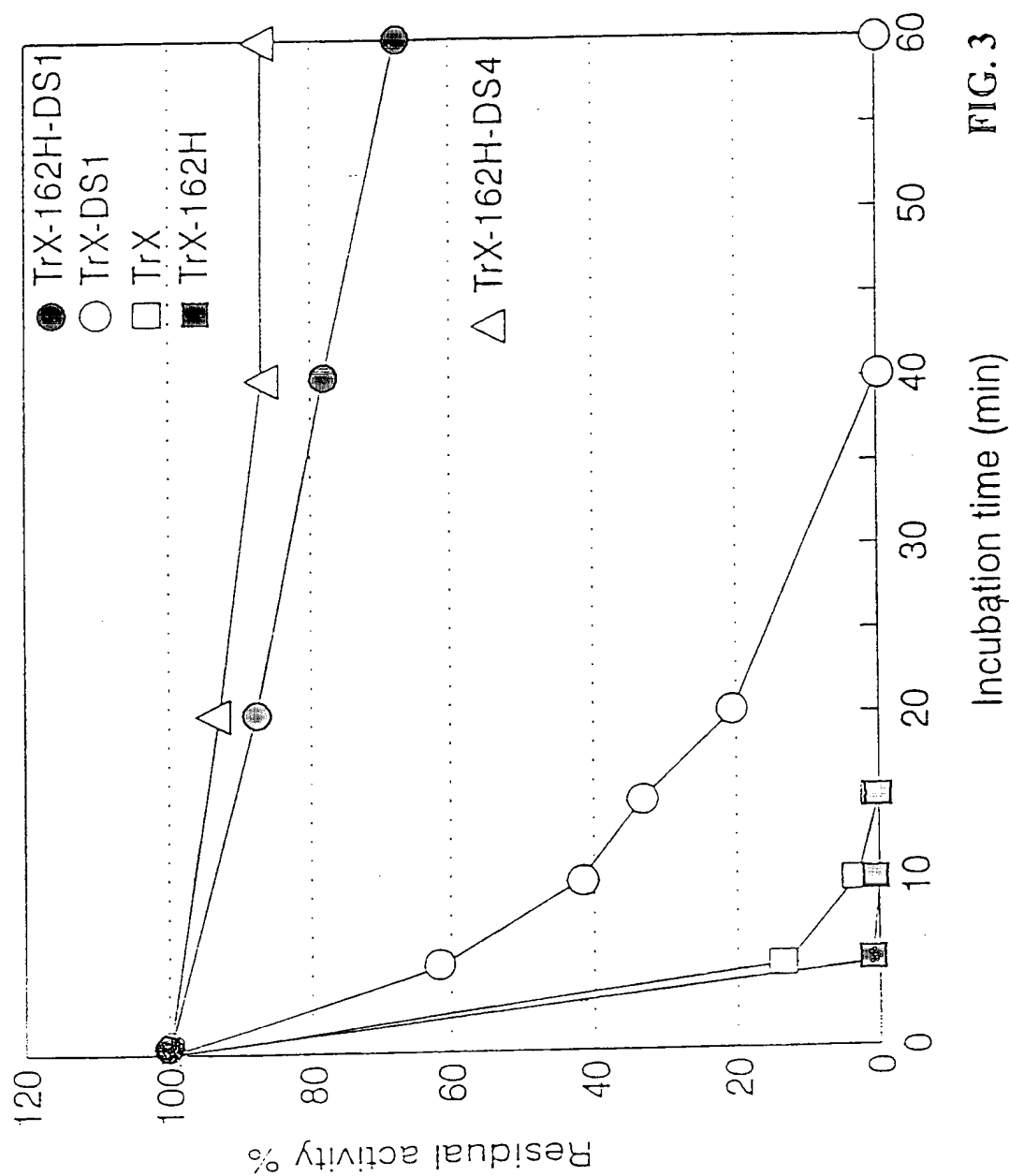


FIG. 3

7/12

Thermostability of mutant Trx

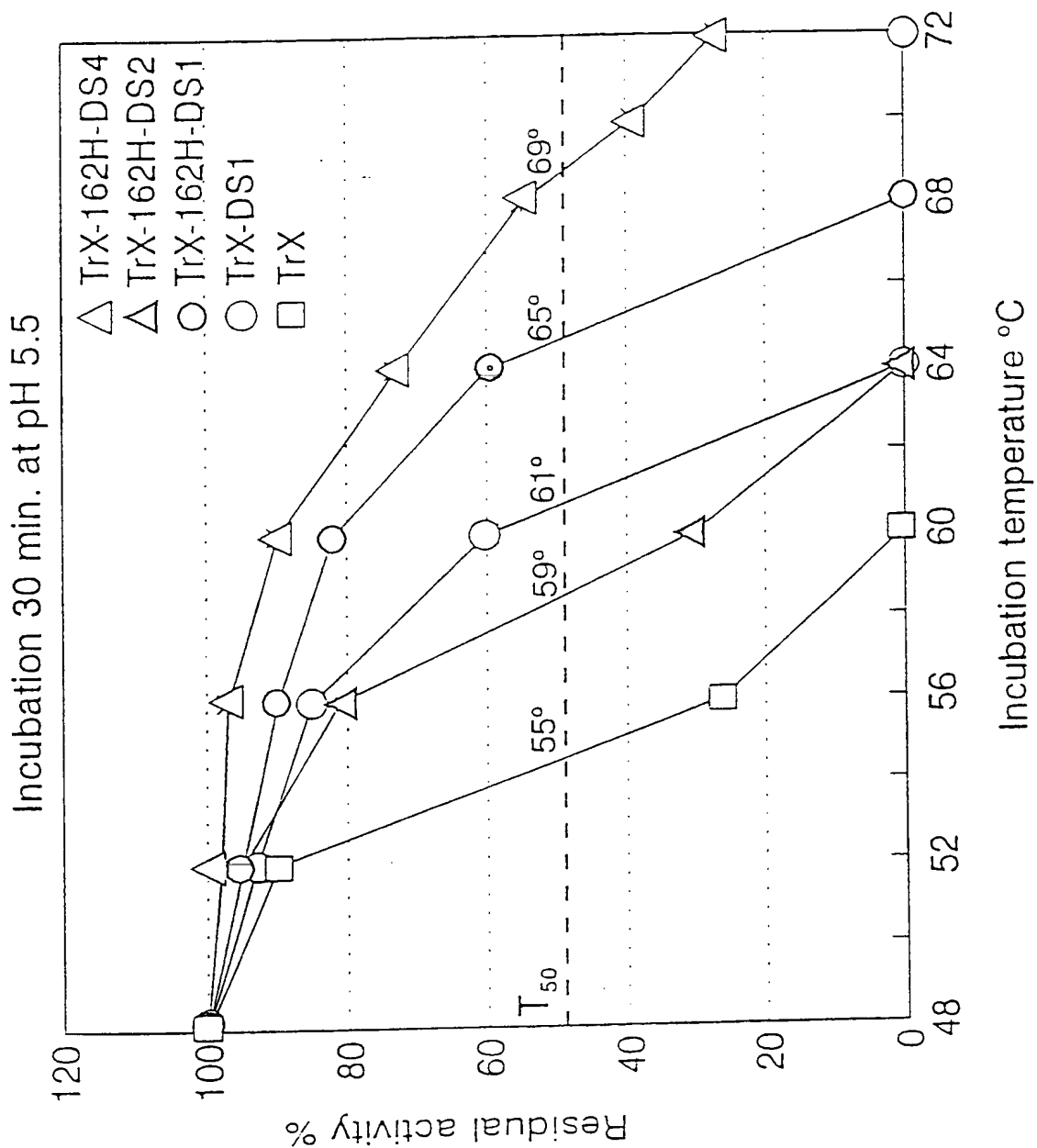


FIG. 4A

8/12

Thermostability of mutant TrX

Incubation 30 min. at pH 5.5

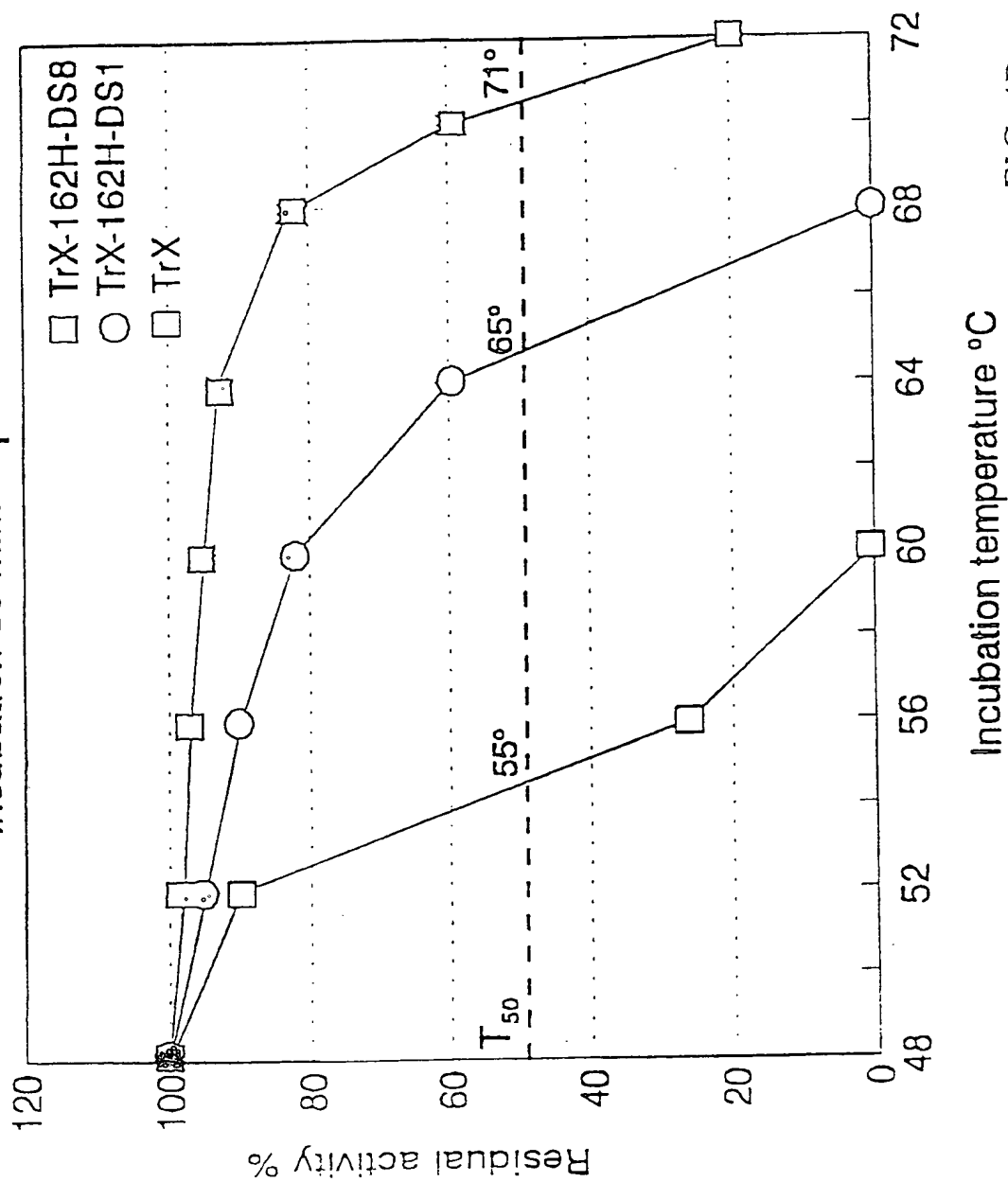


FIG. 4B

9/12

Thermostability of mutant TrX incubation in 40% glycerol, 30 min

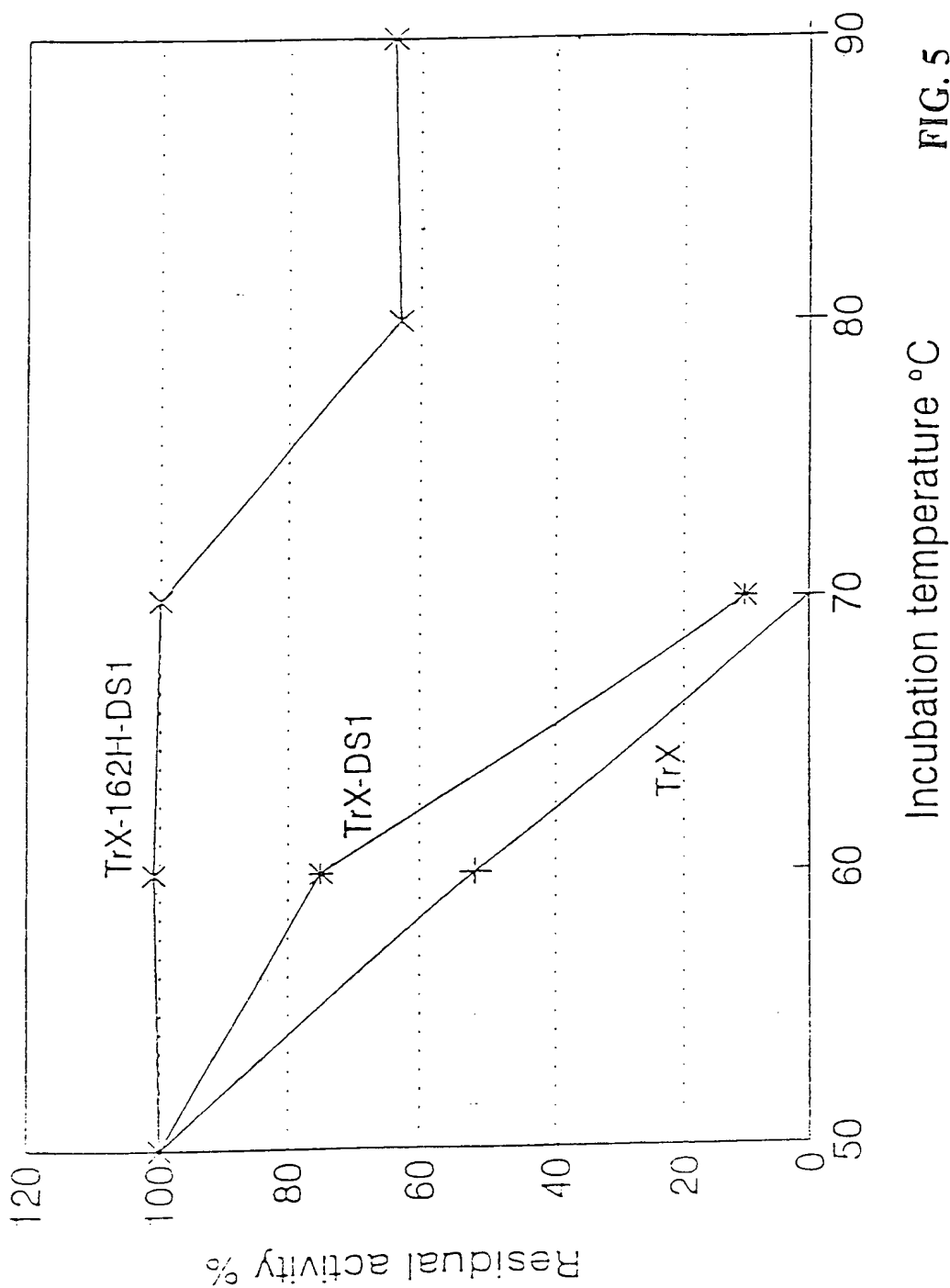


FIG. 5

10/12

Thermostability of TrX-162H-DS1 at 90°C

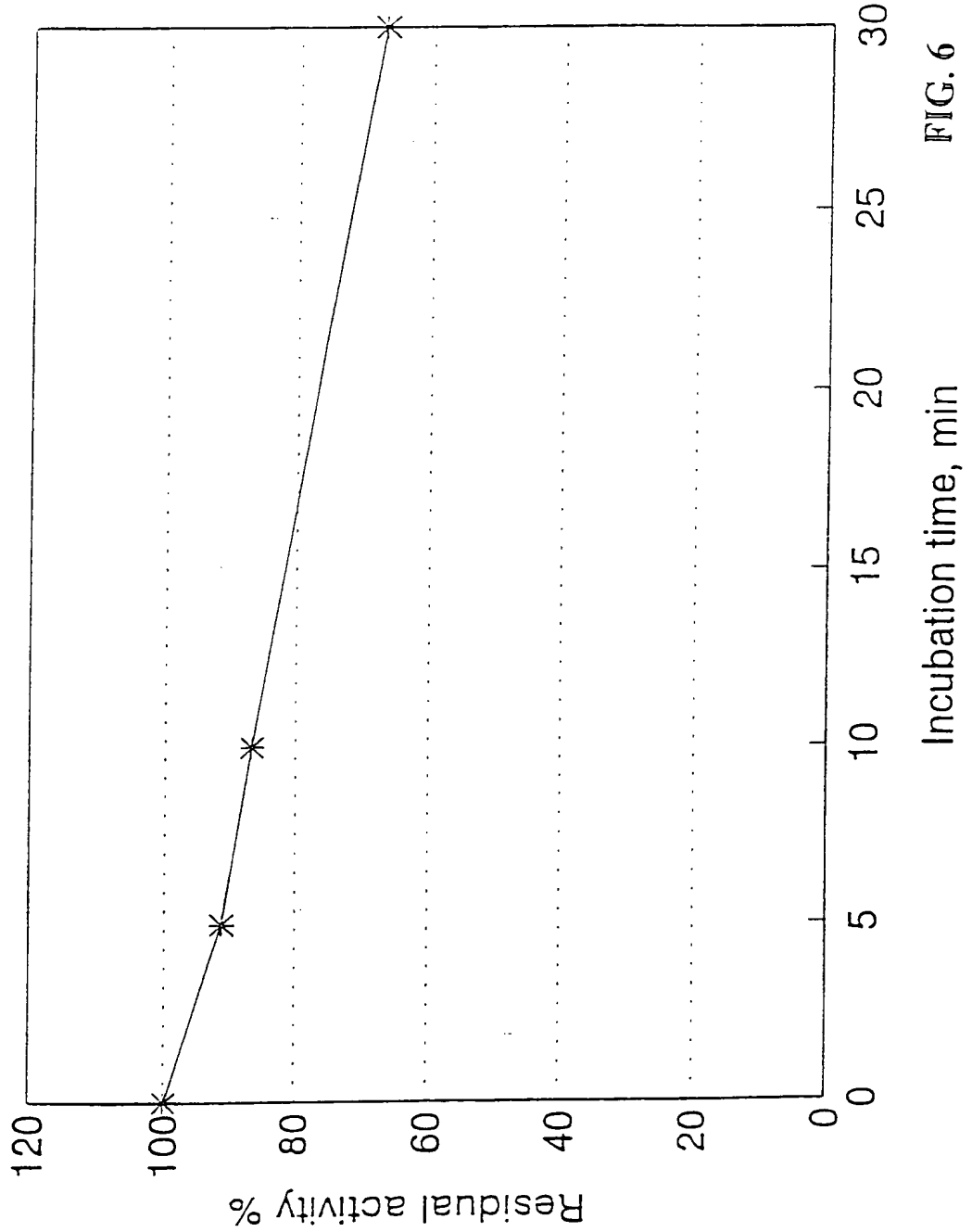


FIG. 6

11/12

Effect of temperature on the hydrolysis of xylan by mutant TrX

pH 4.5, 30 min, 1.5 microgram of each enzyme

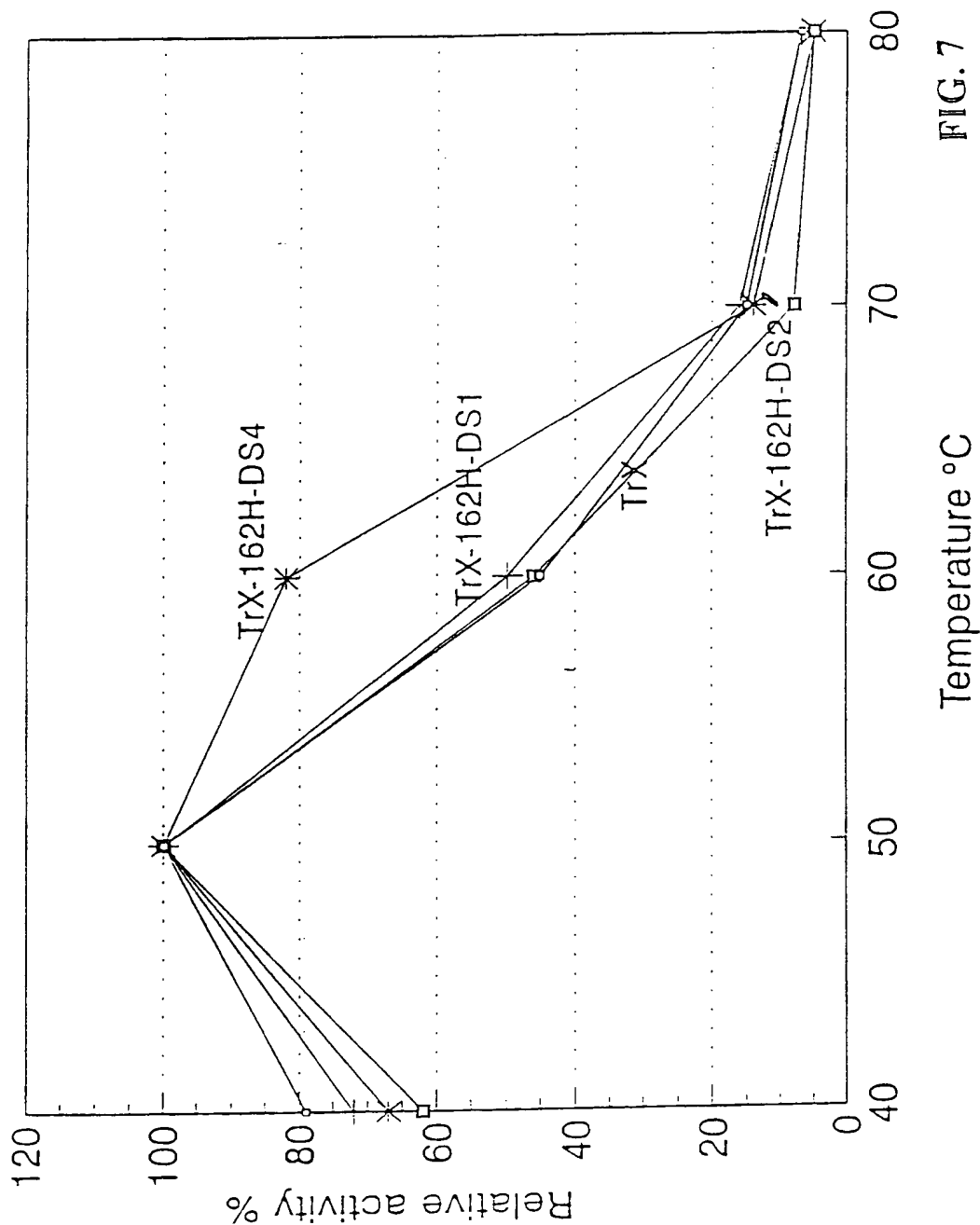


FIG. 7

12/12

